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Effect of home cooking on the antioxidant capacity of vegetables: Relationship with Maillard reaction indicators

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ABSTRACT

Vegetables are health-promoting foods due to their content on a wide range of phytochemicals, being involved in antioxidant protection. However, such bioactivity can be modified during cooking and also along the digestion-fermentation process. Thus, the aim of the paper is to establish a relation among the type of processing (raw, boiled, steamed, grilled, roasted, and fried), time of processing (raw, usual time and well-done), antioxidant capacity and the development of the Maillard reaction (measured though the analysis of furosine and HMF) of 23 widely consumed vegetables. Antioxidant capacity was measured with three methods ($TEAC_{ABTS}$, $TEAC_{FRAP}$, $TEAC_{OH}$) after submitting vegetables to an *in vitro* digestion followed by and *in vitro* fermentation process. Furosine and HMF were useful indicators to control both cooking time and heat intensity of common vegetables, being correlated with antioxidant capacity. Those samples cooked with aggressive techniques (frying, grilling or breading) showed the higher antioxidant values.

1. Introduction

Vegetables and fruit consumption is strongly linked to a lower risk of several chronic diseases and according to dietary guidelines (Liu, 2013). Vegetables provide a wide range of phytochemicals involved in antioxidant protection (Abuajah, Ogbonna, & Osuji, 2015; Liu, 2013). All these compounds have been positively related to chronic diseases such as cancer, diabetes type II, atherosclerosis, etc. (Abuajah et al., 2015).

Most vegetables are consumed after being cooked under different conditions, involving water or oil mediums and a wide range of temperatures. Thus, the heat treatment will become very important since it will affect directly the final composition of vegetables. Several cooking methods are widely used in vegetables depending on the temperature (Bello Gutierrez, 1998); boiling and steaming are less aggressive, compared to frying, roasting or grilling. On the other hand, the cooking medium (water or oil) is also important since the use of boiling water could decrease the concentration of certain hydrosoluble compounds, whereas the use of oil can increase their antioxidant capacity due to an improvement of the phytochemicals content (Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir, & de la Serrana, 2015). However, thermal treatment can also improve bioactive compounds availability by braking down cell structures (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008). Accordingly, the phytochemical composition of vegetables changes during culinary processing (Bunea et al., 2008; Podsędek, 2007; Soares, Carrascosa, & Raposo, 2017).

The Maillard reaction is also a source of compositional changes in vegetables during cooking, since the carbonyl group of a reducing sugar or other molecule with carbonyl groups (vitamin C, oxidized lipids, etc.) reacts with the free amino group of a protein, amino acid or peptide (de la Cueva, Seiquer, Mesías, Rufián-Henares, & Delgado-Andrade, 2017). Both processing time and temperature are important variables to take into account for the development of the Maillard reaction. This reaction is favored at temperatures above 50 °C and long cooking times (Rufián-Henares & de la Cueva, 2008). Thus, aside from the cooking technique used, it is important to establish the effect of processing time. Therefore, an aggressive long cooking procedure could result in a loss of bioactive compounds (Rufián-Henares, Guerra-Hernández, & García-Villanova, 2013). In order to control the development of the Maillard reaction, different chemical indicators have been used during thermal processing in food manufacturing companies (Rada-Mendoza, García-Baños, Villamiel, & Olano, 2004). In this sense, furosine is a product of the acidic hydrolysis of Amadori compounds, and has been used as an indicator of the initial stages of Maillard reaction, where organoleptic changes are still not present. On the other hand, 5-hydroxymethylfurfural (HMF) is produced during intermediate

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stages of Maillard reaction and therefore used as an indicator of middle advanced Maillard reaction (Rufián-Henares, García-Villanova, and Guerra-Hernández (2008). Accordingly, furosine and HMF have been used for evaluating the intensity of heating in several vegetable products such as onion, garlic, potato, carrots, among others (Rufián-Henares et al., 2008; Rufián-Henares et al., 2013). There are also several papers which study the antioxidant properties of Maillard reaction products from several sources (Carvalho, Correia, Lopes, & Guido, 2014; de la Cueva et al., 2017; Dittrich et al., 2009; Pastoriza & Rufián-Henares, 2014). In this sense, vegetable processing could increase antioxidant capacity even after losing some phytochemicals due to either loss in boiling water or to heat during processing.

Therefore, the main objective of this paper is to establish a relation among the type of processing (raw, boiled, steamed, grilled, roasted, and fried), time of processing (raw, usual time and well-done), antioxidant capacity and the development of the Maillard reaction (measured through the analysis of furosine and HMF) of 23 widely consumed vegetables. Antioxidant capacity will be measured after submitting vegetables to an *in vitro* digestion followed by and *in vitro* fermentation process, in order to mimic as much as possible physiological conditions. The main novelty of this work is not the relation among antioxidant capacity and the cooking technique, but the effect that thermal damage (monitored through furosine and HMF determination) has over antioxidant capacity. Another novelty is the use of an *in vitro* digestion-fermentation process to better simulate the physiological extraction and transformation of bioactive compounds. This would provide valuable information about how culinary treatments and their degree of intensity can affect antioxidants available at the small and large intestine.

2. Materials and methods

2.1. Chemicals

Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), potassium persulphate, sodium hydroxide, hydrochloric acid, iron (III) chloride hexahydrate, sodium acetate, potassium chloride, potassium di-hydrogen phosphate, sodium mono-hydrogen carbonate, sodium chloride, magnesium chloride hexahydrate, ammonium carbonate, calcium chloride dihydrate, sodium di-hydrogen phosphate, tryptone, cysteine, sodium sulphide, resazurin, salivary alpha-amylase, pepsin from porcine, bile acids (porcine bile extract), ethanol, hydrochloric acid, acetonitrile, and HMF standard were from Sigma-Aldrich (Darmstadt, Germany). Pancreatin from porcine pancreas was purchased from Alpha Aesar (United Kingdom). Furosine standard from Neosystem Laboratories (Strasbourg, France).

2.2. Vegetable samples and cooking conditions

Fresh chard (*Beta vulgaris* subsp. *Vulgaris)*, garlic *(Allium sativum)*, artichoke (*Cynara cardunculus* var. *scolymus*), eggplant (*Solanum melongena*), zucchini (*Cucurbita pepo*), pumpkin (*Curcubita moschata*), onion (*Allium cepa* L.), mushroom (*Agaricus bisporus*), cabbage (*Brassica olaracea* var. *gemmifera*), cauliflower (*Brassica olaracea* var. *botrytis*), asparagus (*Asparagus oficinalis*), spinach (*Spinacia oleracea*), peas (*Pisum sativum*), broad beans (*Vicia faba*), beans (*Phaseolus vulgaris*), lettuce (*Lactuca sativa*), potato (*Solanum tuberosum*), cucumber (*Cucumis sativus*), parsley (*Petroselinum crispum*), red pepper (*Capsicum annuum*), leek (*Allium ampeloprasum* var. *porrum*), tomato (*Solanum lycopersicum*) and carrot (*Daucus* carota) were purchased in local markets. Vegetables were washed and, peeled when applicable. Each one of them was submitted to the usual culinary processes common for each vegetable (**Table S1**) following traditional recipes. Vegetables were cut in different sizes to achieve the same texture in the same cooking time. Carrots, zucchini, pumpkin, onion, red pepper, asparagus and leek were cut in fine dices (brunoise cutting). Green leaf vegetables were shredded (chiffonade cutting). Eggplant was cut in sticks (julienne cutting). Potatoes, tomato and cucumber were cut to obtain broad and thin slices (parallel cutting). Garlic was crushed and parsley was minced. Additionally, for each culinary process, two processing times were applied; the usual one (normal; N) and a longer processing time consisting on a 50% more of exposure (well done; WD). The culinary treatments chosen were steaming, water boiling, grilling, roasting, frying and breading. Extra virgin olive oil (EVOO) was used as medium for grilling and frying. Steaming and boiling were carried out at 100 °C for 20 min (N) and 30 min (WD) with a proportion water:vegetable in the case of boiling of 5:1. Grilling was carried out at 220–250 °C for 3 min on each side (N) and 4.5 min each side (WD) with a proportion oil:vegetable of 0.5:1. Roasting was carried out at 180 °C for 10 min (N) and 15 min (WD). Frying and breading were carried out at 180 °C for 8 min (N) and 12 min (WD) with a proportion oil:vegetable of 5:1. Breaded vegetables were covered with flour prior to be fried. The same amount of flour was used for each vegetable. The utensils used for sample preparation were the following: stainless steel spoons, forks, and knives, frying pan, saucepan, household size steam cooking machine and a portable oven (1500 W). All these utensils were purchased from Centro Hogar Sanchez (Granada, Spain). Cooking times and medium proportions were acquired from Ramírez-Anaya et al. (2015) and adapted to our own equipment and laboratory conditions. Samples were homogenized and stored under nitrogen atmosphere at −80 °C in order to avoid oxidations. All analyses were carried out in triplicate.

2.3. In vitro digestion

All vegetables were subjected to an *in vitro* digestion process followed by an *in vitro* fermentation to mimic physiological processes in the human gut. The *in vitro* digestion method was carried out according to the protocol described by Pérez-Burillo, Rufián-Henares, and Pastoriza (2018a). The gastrointestinal *in vitro* digestion was composed of an oral phase (5 min at 37 °C with alpha-amylase 75 U/mL, pH 7.0), a gastric phase (2 h at 37 °C with pepsin 2000 U/mL at pH 3.0) and an intestinal phase (2 h at 37 °C with pancreatin 13.37 mg/mL at pH 7.0).

2.4. In vitro fermentation

The *in vitro* fermentation was carried out according to the protocol described by Pérez-Burillo et al. (2018a). *In vitro* fermentation was carried out using faecal samples from three healthy donors (not taking antibiotics, people with body mass index within the “normal weight range”, mean Body Mass Index = 21.3). The solid residue obtained after *in vitro* gastrointestinal digestion plus 10% of the digestion supernatant was fermented (500 mg).

After *in vitro* gastrointestinal digestion and *in vitro* fermentation three different fractions were obtained: digestion supernatant (fraction available for absorption at the small intestine), fermentation supernatant (fraction available for absorption at the large intestine) and fermentation solid residue (fraction not available for absorption and excreted with feces).

2.5. Antioxidant capacity

The antioxidant capacity of three fractions was assessed: the supernatant obtained after gastrointestinal digestion, the supernatant derived from fermentation and the solid residue remaining after fermentation. Three different methods were used to analyse the antioxidant capacity of foods. All antioxidant capacity values for all three methods were corrected taking into account their respective blanks (enzymes, chemicals and inoculum).

$TEAC_{OH}$ method: In this method, performed to unravel the scavenging activity against OH· radicals, carmin indigo was used as the redox indicator, following the method of Pérez-Burillo, Rufián-Henares, and

Pastoriza (2018b). It is carried out at physiological pH (7.24). The results obtained are expressed as mmol Trolox equivalents per kg of sample.

$TEAC_{ABTS}$ assay: This method measures the activity of the samples against ABTS· radicals. The ABTS assay was conducted as described by Re et al. (1999) with slight modifications. Results are expressed as mmol equivalents of Trolox per kg of sample.

$TEAC_{FRAP}$ assay: The ferric reducing ability of the extract of each sample was estimated following the procedure described by Benzie and Strain (1996) with minor modifications. Results are expressed as mmol equivalents of Trolox per kg of sample (Benzie & Strain, 1996).

2.6. Furosine assay

Furosine determination was performed following the method described by Rufián-Henares et al. (2013). The analysis was performed in duplicate and the results are expressed as mg of furosine/kg of sample.

2.7. HMF assay

HMF determination was performed following the method described by Rufián-Henares et al. (2008). The analysis was performed in duplicate and the results are expressed as mg of HMF/kg of sample.

2.8. Sugars and protein content

Sugars and protein content of all 23 vegetables were taken from the food composition database of the United States Department of Agriculture (USDA), available at https://ndb.nal.usda.gov/ndb/search/list?home=true.

2.9. Statistical analysis

The statistical significance of the data was tested by one-way analysis of variance (ANOVA), followed by the Duncan test to compare the means that showed significant variation ($p < 0.05$). As factors for ANOVA we used type of cooking (frying, grilling, roasting, boiling, steaming, breading, and raw) and intensity of the cooking (raw, normal time or extended time [WD]). Statistical analysis was performed using raw vegetables as the reference group. Pearson correlation coefficient was calculated to show the lineal relation between antioxidant capacity and furosine at a p value < 0.05. All the statistical analyses were performed using Statgraphics Plus software, version 5.1.

3. Results and discussion

3.1. Furosine and HMF content

Furosine and HMF content were analyzed in 127 vegetable samples submitted to different cooking techniques (breaded, fried, grilled, roasted, boiled, steamed and raw) for two different periods of time; one of them would be the time described in traditional recipes (normal, N) and the other one involving cooking for a 50% more time to obtain the commonly known as “well done” form (WD). Therefore, it is possible to distinguish among type of treatment and intensity of the treatment (**Table S1**). Furosine and HMF, two sensitive markers of Maillard reaction, were used to unravel heat damage during vegetables cooking (Fig. 1). The vegetables that showed higher furosine content were red pepper and eggplant, followed by artichoke and cauliflower (**Table S2).** In the case of HMF, these vegetables were eggplant followed by red pepper, cauliflower and onion (**Table S2).** Regarding the type of treatment (Fig. 1A and C), breaded and fried vegetables showed the highest furosine and HMF values (86.3 and 82.1 mg of furosine/Kg sample, respectively and 140.8 and 50.1 mg of HMF/Kg sample, respectively), which is a logical result since the high temperature used during frying produce greater thermal damage due to the Maillard reaction. All culinary techniques but steaming showed significantly higher furosine content than the raw form ($p < 0.05$). However, HMF was not detected in boiled, steamed and raw vegetables, probably because HMF is an indicator of intermediate Maillard reaction stages and therefore such cooking techniques did not provide enough energy to develop the reaction further. These results are in accordance with those of other authors (Delgado-Andrade, Seiquer, Haro, Castellano, & Navarro, 2010). In the case of breaded vegetables, the combination of the high temperatures used for frying and the presence of flour (high starch content) resulted in the highest values of furosine and especially of HMF. Grilled vegetables and roasted vegetables were both submitted to similar temperatures (around 200 °C) and still their furosine and HMF values are much lower than in fried vegetables. The reason behind could be that, in the case of roasting, heat is transmitted through the air not having direct contact with the vegetable (resulting in a lower damage). On the other hand, grilled vegetables are in direct contact with the source of heat, but as a consequence, they are exposed to the heat source for a shorter period of time in comparison to frying. In this case, the use of high temperature (180 °C) and a liquid medium (olive oil) for heat transfer give rise to a high thermal damage.

Regarding treatment intensity (Fig. 1B and D), WD vegetables had around twice as much furosine as N vegetables (37.2 *Vs.* 16.9 mg of furosine/Kg sample, respectively). In the case of HMF, raw vegetables did not show any HMF content whereas WD vegetables content was 28.3 mg of HMF/Kg sample and N vegetables was 8.1 mg of HMF/Kg sample. N and vegetables showed significantly higher furosine values than raw ones ($p < 0.05$) whereas WD vegetables showed significantly higher values of furosine and HMF than N vegetables. These results make sense since the Maillard reaction is favored by time and temperature (Rufián-Henares et al., 2008), concluding that overcooking vegetables could result in a greater development of Maillard reaction. However, though Maillard reaction development is also related to food composition (reducing sugars and protein content), in our case furosine and HMF content can be explained mainly by the type of treatment and intensity. The content of sugars (2.62 ± 1.48 g/100 g) and proteins (2.91 ± 4.63 g/100 g) are not exactly the same in all samples, but these differences seem to be insufficient to play a predominant role in Maillard reaction advance compared to the cooking technique. This conclusion is supported by the absence of statistically significant correlations between furosine or HMF and sugar (or protein content) in every foodstuff and type of treatment. Accordingly, since furosine and HMF can be used as an indicator of thermal damage (Delgado-Andrade et al., 2010), for the ulterior multivariable analysis the culinary techniques were classified in descendent order regarding their thermal damage: breaded > fried > grilled > roasted > boiled > steamed > raw.

3.2. Antioxidant capacity

For each sample, the antioxidant capacity of three fractions was assessed: the supernatant obtained after gastrointestinal digestion (which would be the antioxidant capacity available for absorption in the small intestine), the supernatant derived from fermentation (which would be the antioxidant capacity available for absorption in the large intestine) and the solid residue remaining after fermentation (which is not absorbed, but could exert some antioxidant protection on the large intestine walls). The sum of the three terms is the total antioxidant capacity (Pérez-Burillo et al., 2018a). Three different methods were used to analyse the antioxidant capacity of foods. All antioxidant capacity values for all three methods were corrected taking into account their respective blanks (enzymes, chemicals and inoculum).

- **Gastrointestinal digestion supernatant**. Regarding $TEAC_{ABTS}$, antioxidant capacity released during in vitro digestion was significantly ($p < 0.05$) higher in all cooking techniques, in comparison to raw vegetables, but in boiling which was not significant

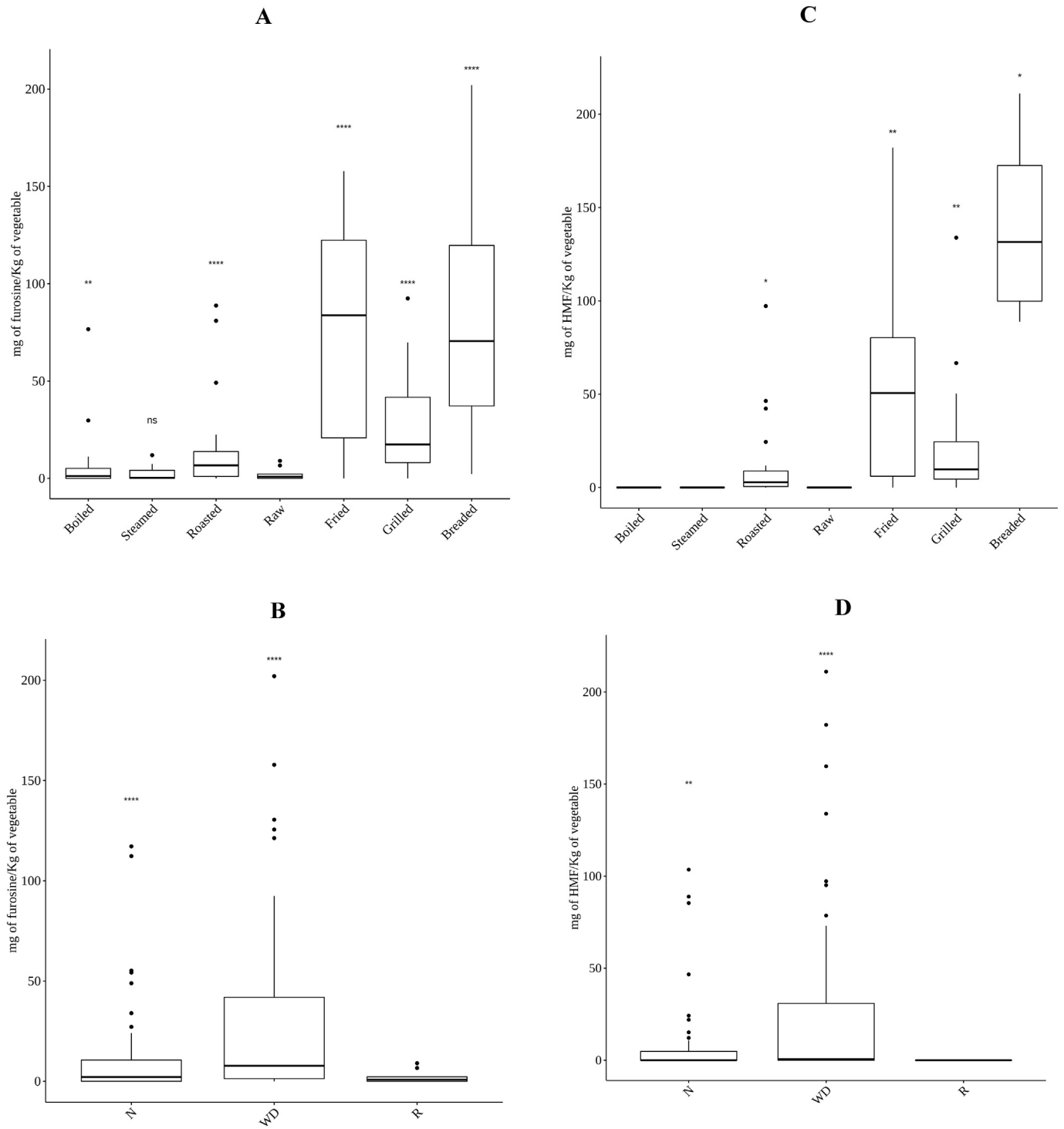

Fig. 1. Furosine content of processed vegetables (mg/Kg of vegetable) depending on the cooking technique (1A) or heat treatment intensity (1B). HMF content of processed vegetables (mg/Kg of vegetable) depending on the cooking technique (1C) or heat treatment intensity (1D). Statistical analysis was performed through ANOVA using raw vegetables as the reference group. Values are the mean value of all vegetables for each cooking technique or treatment intensity. Statistic labels: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$, ns: not significant.

(Fig. 2A). Regarding the degree of intensity, N and WD vegetables showed significantly higher antioxidant capacity than raw vegetables ($p < 0.05$) (Fig. 2B). On the other hand, $TEAC_{FRAP}$ assay showed also that antioxidant capacity of digestion fraction was significantly ($p < 0.05$) higher in fried, grilled and breaded vegetables in comparison with the raw ones. However, boiling, steaming and roasting did not increase antioxidant capacity significantly in comparison with the raw form (Fig. 3A). Moreover, WD vegetables showed to be significantly more antioxidant than the raw form (Fig. 3B). $TEAC_{OH}$, as in $TEAC_{FRAP}$, only showed significantly higher antioxidant values than raw vegetables when they were fried, grilled or breaded (Fig. 4A). Moreover, WD vegetables exerted higher antioxidant capacity than raw ones (Fig. 4B). The explanation behind these findings could be that thermal treatments (cooking) could break down cell structures making easier their digestion and therefore releasing more bioactive compounds available for

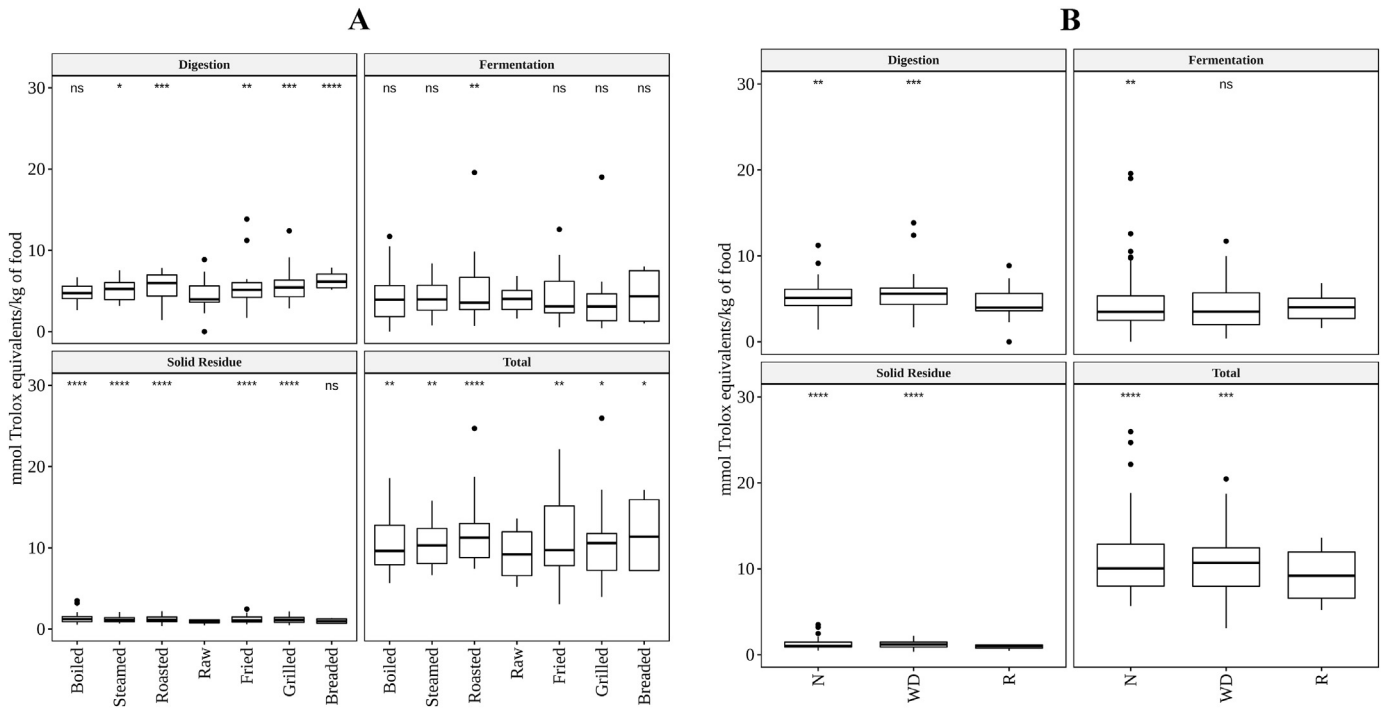

Fig. 2. Antioxidant capacity measured by the $TEAC_{ABTS}$ assay of each fraction depending of each cooking technique (2A) or heat intensity (2B). Statistical analysis was performed through ANOVA using raw vegetables as the reference group. Values are the mean value of all vegetables for each cooking technique or treatment intensity. Statistic labels: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$, ns: not significant.

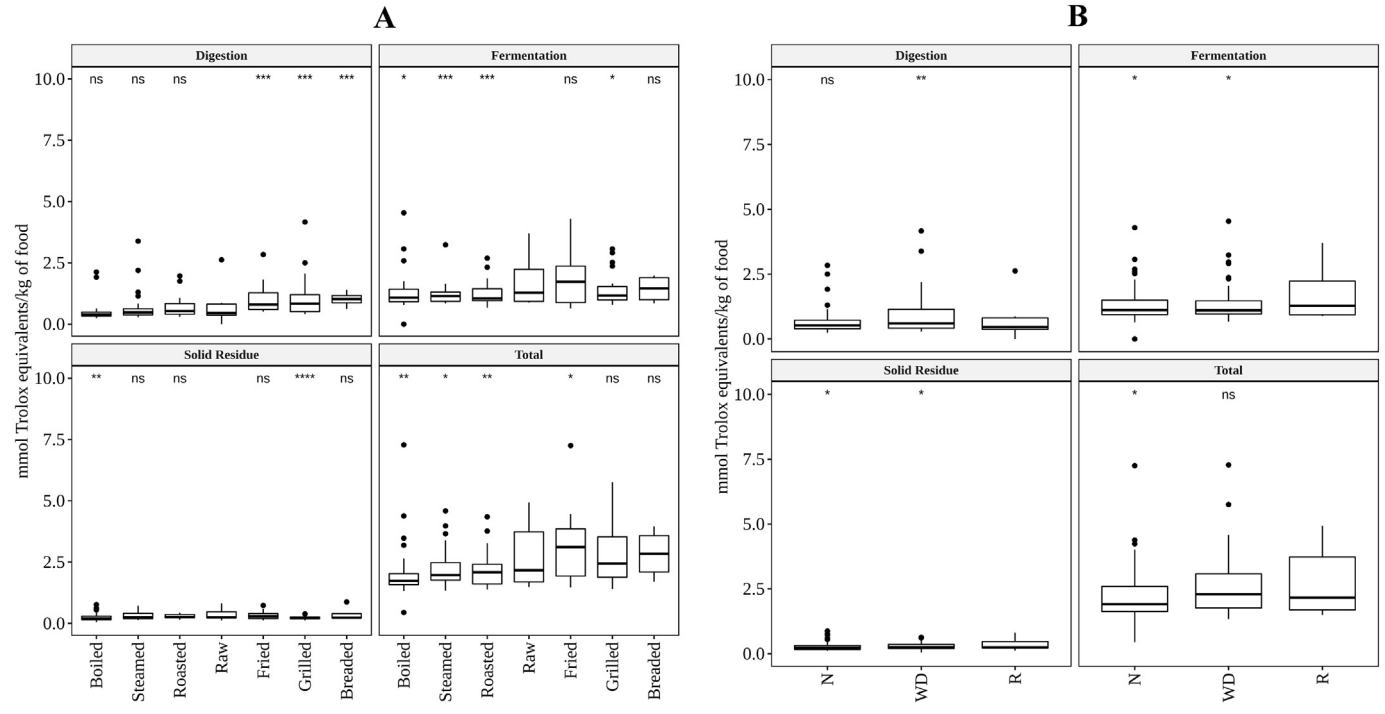

Fig. 3. Antioxidant capacity measured by the $TEAC_{FRAP}$ assay of each fraction depending of each cooking technique (3A) or heat intensity (3B). Statistical analysis was performed through ANOVA using raw vegetables as the reference group. Values are the mean value of all vegetables for each cooking technique or treatment intensity. Statistic labels: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$, ns: not significant.

absorption in the small intestine (Miglio et al., 2008). In the case of $TEAC_{ABTS}$, boiling did not increase antioxidant capacity in comparison with the raw vegetables, which could be due to a solubilization of hydrosoluble compounds in the boiling water (Ramírez-Anaya et al., 2015). However, $TEAC_{FRAP}$ and $TEAC_{OH}$, only showed higher antioxidant capacity when vegetables were fried, grilled or breaded. Accordingly, even though cooking could help releasing antioxidant compounds during digestion, olive oil could play a more important role (Ramírez-Anaya et al., 2015). Additionally, this could mean that reducing compounds and compounds active against

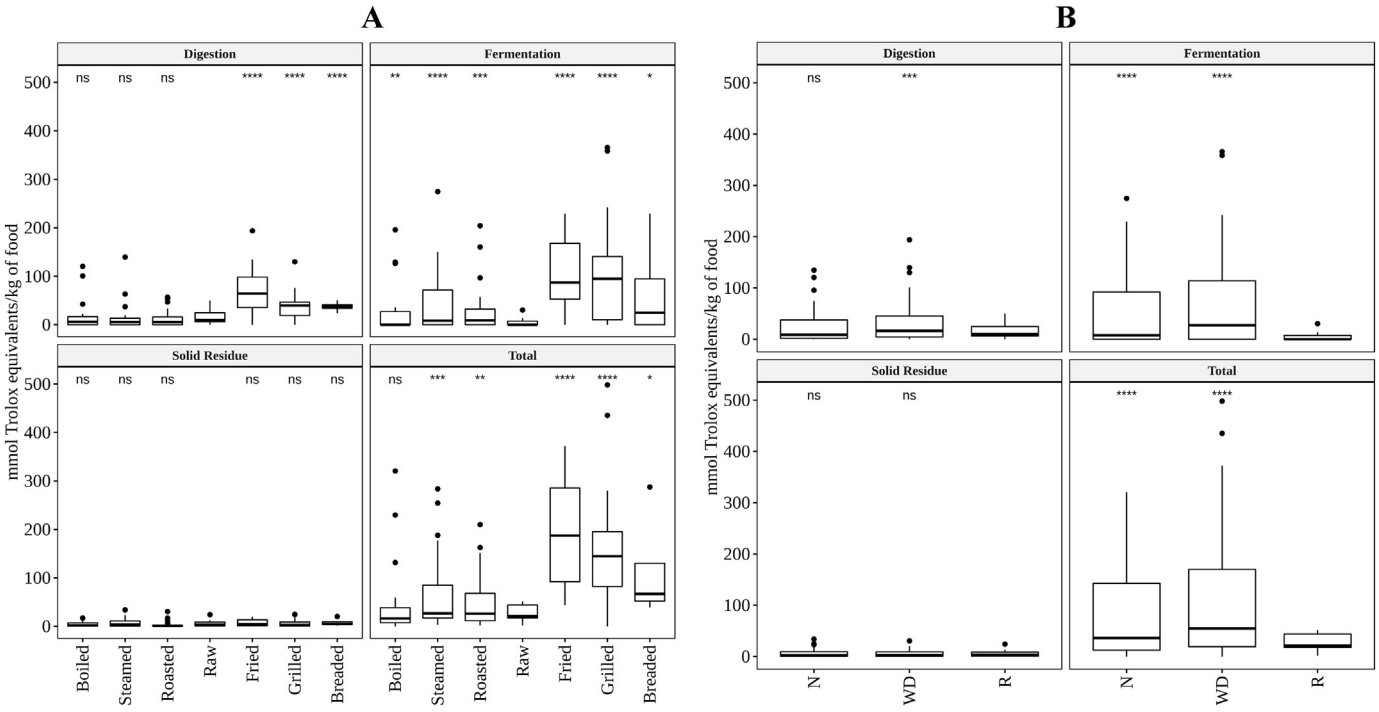

Fig. 4. Antioxidant capacity measured by the $TEAC_{OH}$ assay of each fraction depending of each cooking technique (4A) or heat intensity (4B). Statistical analysis was performed through ANOVA using raw vegetables as the reference group. Values are the mean value of all vegetables for each cooking technique or treatment intensity. Statistic labels: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$, ns: not significant.

OH· radicals would only increase during digestion by adding olive oil. Nevertheless, thermal treatment could also help releasing bioactive compounds as WD vegetables were significantly more antioxidant than raw ones. N cooked vegetables were also more antioxidant but not significantly.

- **Fermentation supernatant**. Regarding $TEAC_{ABTS}$, only roasting increased antioxidant capacity in a significant manner with respect to the raw form ($p < 0.05$) during fermentation (Fig. 2A). N cooked vegetables showed also significantly ($p < 0.05$) higher antioxidant capacity than raw ones (Fig. 2B). In the case of $TEAC_{FRAP}$, fermentation supernatant antioxidant capacity of the raw form was significantly higher than all cooking techniques except for frying and breading which were not significant (Fig. 3A). Moreover, N and WD vegetables showed significantly higher antioxidant capacity than raw ones (Fig. 3B). Regarding $TEAC_{OH}$, each type of culinary technique produced higher antioxidant capacity than raw vegetables during fermentation, especially fried, grilled and breaded ones (Fig. 4A). N and WD vegetables also gave significantly higher antioxidant capacity than raw ones (Fig. 4B). In this step, gut microbial activity could play an important role (Pérez-Burillo et al., 2018b). As results show, according to $TEAC_{ABTS}$, there is no differences between most cooking techniques, which could indicate that the antioxidants active against ABTS· radicals released by gut microbial activity are not affected by the type of cooking. However, in the case of $TEAC_{FRAP}$, we see how reduction capacity released after gut microbial activity could be improve by adding olive oil. Otherwise, raw vegetables produce more bioactive compounds with reduction power than cooked ones. Finally, the activity against OH· released after microbial degradation of vegetables, greatly improves when they are cooked. This could be due to the metabolization of bioactive compounds from the vegetables that reach the colon in larger amounts thanks to an easier digestion but also to the degradation of melanoidins or other compounds that appear during the Maillard reaction.
- **Fermentation solid residue**. Regarding $TEAC_{ABTS}$, antioxidant capacity of the solid residue was significantly higher in all cooking techniques but in breading in comparison with the raw form ($p < 0.05$) (Fig. 2A). N and WD were also significantly more antioxidant than raw vegetables (Fig. 2B). $TEAC_{FRAP}$ on the other hand, did not show significant differences regarding cooking techniques but in the case of boiling and grilling which were significantly lower than the raw form (Fig. 3A). N and WD were also significantly less antioxidant than the raw ones (Fig. 3B). In the case of $TEAC_{OH}$, no significant differences were found neither in relation to cooking techniques nor degree of cooking. The differences found here between $TEAC_{ABTS}$ and $TEAC_{FRAP}$ could be due to the different nature of the methods and the residue left after fermentation could be more active against ABTS· radicals.
- **Total antioxidant capacity and contribution of each fraction to total antioxidant capacity**. **Table S2** in supplemental information shows the antioxidant capacity values for each foodstuff per type of treatment and intensity. For $TEAC_{ABTS}$, he most antioxidant vegetable (mean of the different culinary treatments and degree of intensity) in this antioxidant assay (**Fig. S1, Table S2**) was spinach whereas the lowest one was cabbage. For $TEAC_{FRAP}$, the most antioxidant vegetable was artichoke, followed by beans and red pepper (**Fig. S1, Table S2**) and the less antioxidant leek. For $TEAC_{OH}$, the most antioxidant vegetal was artichoke and the less antioxidant was cucumber (**Fig. S1, Table S2**).

Tables S3 and **S4** show the contribution of each fraction in each sample to total antioxidant capacity. Overall, digestion contribution to total antioxidant capacity was lower in boiled, steamed, roasted and raw vegetables whereas fermentation supernatant contribution was higher in such techniques. The solid residue contribution was also higher in boiled, steamed, raw and fried vegetables (Fig. 5**A** and **B**). Accordingly, taking the above information into account about the antioxidant capacity of cooked vegetables, it could be concluded that during digestion, antioxidant compounds are more easily released from those vegetables that have suffered greater thermal damage, whereas fermentation contributes with higher antioxidant capacity when vegetables are boiled, steamed, roasted or raw (less thermally damaged).

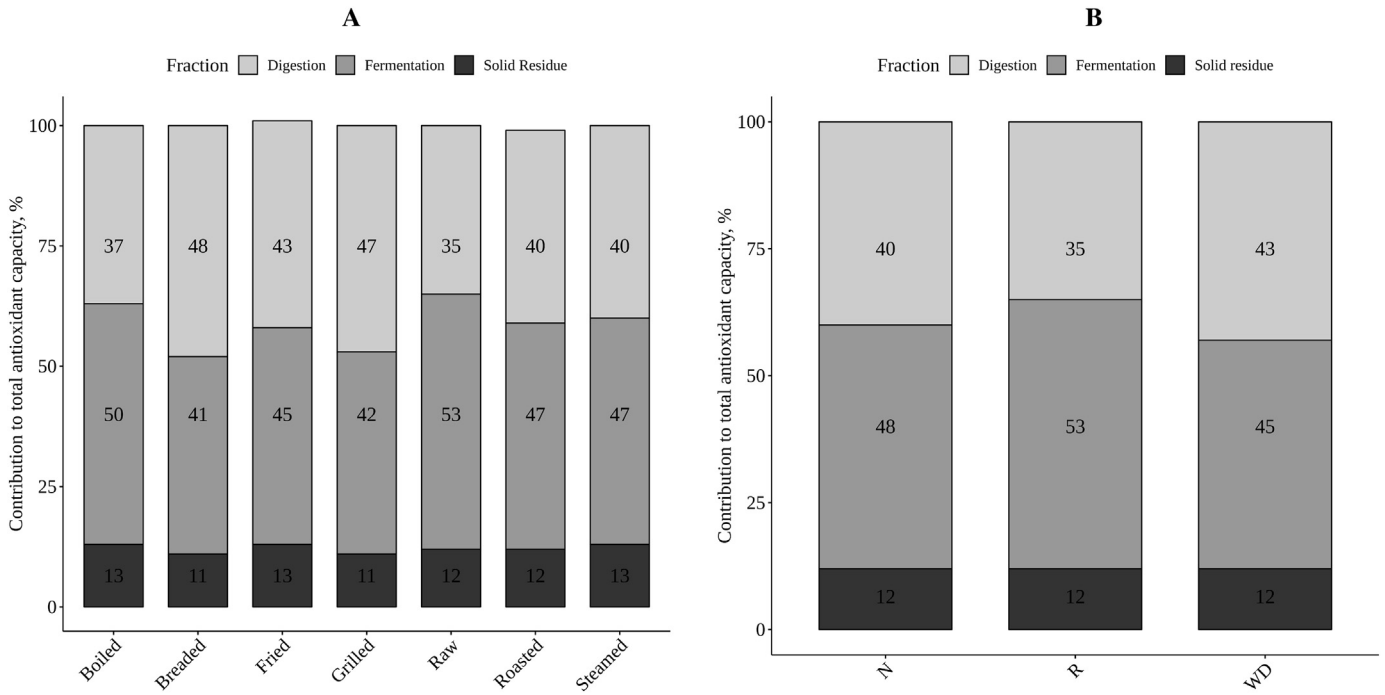

Fig. 5. Contribution to the total antioxidant capacity of the fractions obtained after digestion depending of each cooking technique (5A) or heat intensity (5B).

Therefore, the cooking technique along with the degree of intensity could modify the substrates that reach the colon, which would influence differentially to gut microbiota. Additionally, for all methods tested those vegetables prepared with EVOO had the higher antioxidant capacity. Moreover, in most cases, cooking the vegetable results in an increase of the antioxidant activity, not only when using EVOO. In this sense, our results are in accordance with that found by other authors (Bellail, Shaltout, Youssef, & Gamal, 2012; Miglio et al., 2008; Ramírez-Anaya et al., 2015). However, it could be also interesting for future research to study the effect that olive oil has on its own on antioxidant capacity after gastrointestinal digestion and microbial fermentation of vegetables. Accordingly, it would be interesting further research to elucidate the prebiotic effect of each type of cooking. Another field for future research could be the study of the specific compounds generated during gastrointestinal digestion, and microbial fermentation (including the ones found in feces) to better explain the differences found between antioxidant capacity methods.

- **Correlations between antioxidant capacity, culinary treatment and thermal indicators** (**Table S5**). The type of culinary treatment (breaded, fried, grilled, roasted, boiled, steamed and raw) correlated positively and significantly ($p < 0.05$) with the antioxidant values from TEA_{ABTS} and $TEAC_{FRAP}$ from digestion, total $TEAC_{FRAP}$, and digestion-fermentation $TEAC_{OH}$ (r from 0.19 to 0.43). Treatment intensity (normal, well done and raw) correlated with digestion $TEAC_{FRAP}$ and $TEAC_{OH}$ (r from 0.17 to 0.21). On the other hand, furosine correlated positively ($p < 0.05$) with $TEAC_{FRAP}$ and $TEAC_{OH}$ from digestion, fermentation and total antioxidant capacity (r from 0.26 to 0.38). Finally, HMF correlated also with digestion and fermentation $TEAC_{FRAP}$ and $TEAC_{OH}$ (r from 0.28 to 0.36). These correlations could support that an aggressive treatment (such as frying of grilling) could improve the availability of some molecules due to cell break down. Another possible explanation could be the development of Maillard compounds, which in turn contribute to the antioxidant capacity of other different types of foods (Carvalho et al., 2014; de la Cueva et al., 2017; Delgado-Andrade & Morales, 2005; Dittrich et al., 2009; Martín et al., 2009; Tagliazucchi, Verzelloni, & Conte, 2008).

3.3. Dietary antioxidants per serving and daily antioxidant intake

Every food consumed has an impact on the overall antioxidant capacity, with the corresponding effect on human health. The antioxidant capacity of foods does not come only from one source but from the synergistic effect of a great number of different molecules such as vitamins, phenolic compounds, Maillard reaction products, molecules generated during digestion or fermentation, etc. (Pastoriza, Delgado-Andrade, Haro, & Rufián-Henares, 2011). Accordingly, the antioxidant capacity coming from the daily diet (including all solid and liquid foods) is called dietary antioxidant capacity (Saura-Calixto, Pérez-Jiménez, & Goñi, 2009). That would refer to the amount of antioxidants, expressed as antioxidant units, that undergoes digestion and fermentation and are susceptible to serve human beings as radicals scavengers. Accordingly, two different calculations could be assessed: first, the daily consumption of a given food (MAPAMA, 2018) along with the antioxidant capacity of such foodstuff allows to calculate the contribution of such item to the daily antioxidant intake; secondly, the usual serving size of each foodstuff (García-Arias & García-Fernández, 2003) along with their antioxidant capacity per gram, allows to calculate the antioxidant capacity per serving size, and the contribution of such serving size to daily antioxidant capacity.

Table 1 shows the contribution to daily antioxidant intake and antioxidant capacity per serving size depending only on the vegetable type (including those vegetables for which consumption data in Spain are available). The contribution to daily antioxidant intake and antioxidant capacity per serving size of each vegetable per culinary technique and degree of intensity is in **Table S6**. In order to perform the calculations, the mean antioxidant capacity intake in Spain was obtained from Saura-Calixto and Goñi (2006): 3549 and 6014 μmol Trolox equivalents/day for the ABTS and FRAP methods, respectively. Regarding the ABTS method, the vegetables with higher contribution to the daily antioxidant intake were potato (18.3%) followed by tomato (10.4%) and onion (5.4%). Although these vegetables were not the most antioxidant ones (garlic, cauliflower or asparagus were more antioxidant) they are the main contributors to daily antioxidant intake due to their high consumption. However, when focusing on the antioxidant capacity per serving size, garlic, mushroom, asparagus and

Table 1
Contribution of vegetables consumption to the daily antioxidant activity (AOX) intake in the Spanish diet.

Vegetable	Daily consumption	AOX	Content	AOX daily intake[a]	Contribution to daily intake	AOX serving intake[b]	Contribution to daily intake
	g/day/person	assay	μmol Trolox/ g	μmol Trolox/day	%	μmol Trolox/ serving	%
Green leaf vegetables	3.84	$TEAC_{ABTS}$	9.90	38.0	1.1	1981	55.8
Garlic	2.74		12.7	34.9	1.0	2547	71.8
Eggplant	4.66		9.06	42.2	1.2	1811	51.0
Zucchini	10.7		8.95	95.6	2.7	1790	50.4
Onion	20.8		9.18	192	5.4	1836	51.7
Mushroom	3.84		12.5	48.1	1.4	2506	70.6
Cabbage	4.93		6.40	32.2	0.9	1308	36.8
Cauliflower	4.93		13.4	67.3	1.9	2728	76.9
Asparagus	1.92		14.5	27.0	0.8	2811	79.2
Beans	6.85		8.15	55.9	1.6	1631	46.0
Lettuce	12.3		11.7	144	4.1	2340	65.9
Potato	62.7		10.4	650	18.3	2074	58.4
Cucumber	6.30		7.45	46.9	1.3	1490	42.0
Red pepper	14.0		8.42	118	3.3	1683	47.4
Tomato	39.2		9.39	368	10.4	1879	52.9
Carrot	9.9		11.2	111	3.1	2241	63.1
Green leaf vegetables	3.84	$TEAC_{FRAP}$	2.49	9.57	0.2	499	8.3
Garlic	2.74		2.46	6.75	0.1	493	8.2
Eggplant	4.66		2.51	11.7	0.2	502	8.3
Zucchini	10.7		2.06	22.1	0.4	413	6.9
Onion	20.8		2.02	42.0	0.7	403	6.7
Mushroom	3.84		2.23	8.56	0.1	447	7.4
Cabbage	4.93		2.35	11.6	0.2	471	7.8
Cauliflower	4.93		2.44	12.0	0.2	488	8.1
Asparagus	1.92		2.59	4.98	0.1	519	8.6
Beans	6.85		2.13	14.6	0.2	426	7.1
Lettuce	12.3		1.55	19.1	0.3	309	5.1
Potato	62.7		1.90	119	2.0	381	6.3
Cucumber	5.63		1.69	10.6	0.2	338	5.6
Red pepper	12.2		3.67	51.3	0.9	734	12.2
Tomato	6.04		1.82	71.1	1.2	363	6.0
Carrot	7.22		2.16	21.3	0.4	432	7.2

[a] Considering consumption for a whole year.
[b] Consideing the complete serving ingested a particular day.

cauliflower showed the higher percentages of contribution (from 70.6% to 79.2%) due to their higher antioxidant capacity per gram. In the case of the FRAP method, the contribution to daily antioxidant intake was also higher in potato, tomato and onion due to their high consumption. However, the larger serving size of red pepper make this food as the most important contributor to the daily intake of reducing capacity.

Table 2 shows the contribution of vegetables to the daily intake of antioxidant capacity depending on the culinary technique and the degree of intensity. In order to perform the calculations, the mean daily consumption of vegetables (171 g/day/person) was taken into account. Focusing on the type of culinary treatment, in the case of the ABTS method breaded, fried and roasted vegetables (from 66.3% to 67.5%) had the highest contributions to the daily antioxidant intake. For the FRAP method fried and breaded vegetables showed the highest percentages of contribution (from 9.4% to 10.7%). Both situations are related to a higher antioxidant capacity per gram of food, as explained in the previous section. On the other hand, when focusing on the degree of intensity, regarding N vegetables had the highest contribution (for the ABTS method) followed closely by well-done vegetables. However, in the case of FRAP, the higher contribution was obtained for raw vegetables, followed very closely by WD vegetables. These differences could be due to a loss of reducing compounds during cooking. However, Maillard reaction compounds could also participate in antioxidant capacity making it higher in WD than N vegetables. ABTS· trapping ability, however, could increase with cooking but decrease if it is done for a longer time.

4. Conclusions

In conclusion, furosine and HMF are useful indicators to control both cooking time and heat intensity of common vegetables. In addition, it has been demonstrated that furosine and HMF correlate with the evolution of antioxidant capacity of vegetables in many different cooking techniques. In this sense, those samples cooked with aggressive techniques (frying, grilling or breading) showed the higher antioxidant values. This could be related with the release of antioxidant compounds both due to degradation of cell structures or the generation of neoformed Maillard reaction products with high antioxidant capacity. Our results suggests that with raw, steamed, boiled or roasted vegetables the substrates that reach the colon are different at some extent than those that come from fried, breaded or grilled vegetables. This fact, as demonstrated with antioxidant capacity, could promote different microbial communities and therefore have some effect on host health. In addition, another plausible reason could be the use of EVOO on such culinary preparations. Finally, it is noteworthy to mention that the GAR + (Global Antioxidant Response after gastrointestinal digestion and microbial fermentation) method allows unravelling the contribution to total antioxidant capacity of different fractions obtained after digestion and fermentation of vegetables. Since the liquid and solid fractions obtained after microbial fermentation exert a high antioxidant capacity, future studies should include the GAR + approach to study the modifications on the bioactivity of food as a first step before animal and human nutritional interventions.

Table 2
Contribution of vegetables consumption (depending on their culinary processing) to the daily antioxidant activity (AOX) intake in the Spanish diet.

Culinary treatment	AOX assay	Content			Daily intake			Contribution to daily intake			Content per serving intake			Contribution to daily intake		
		μmol Trolox/g			μmol Trolox/day[a]			%			μmol Trolox/serving[b]			%		
		min	mean	max	min	mean	max	min	mean	max	min	mean	max	min	mean	max
Boiled	$TEAC_{ABTS}$	5.7	10.6	18.6	970	1804	317	27.3	50.8	89.4	1137	2114	3717	32.0	59.6	105
Breaded		7.2	11.8	17.1	1231	2008	2924	34.7	56.6	82.4	1442	2353	3426	40.6	66.3	96.5
Fried		3.1	11.8	22.2	525	2021	3781	14.8	57.0	107	616	2367	4431	17.3	66.7	125
Grilled		4.0	10.4	26.0	676	1776	4430	19.1	50.0	125	793	2081	5191	22.3	58.6	146
Raw		5.2	9.3	13.6	892	1581	2323	25.1	44.6	65.5	1045	1853	2722	29.4	52.2	76.7
Roasted		7.4	12.0	24.7	1268	2043	4215	35.7	57.6	119	1485	2394	4939	41.8	67.4	139
Steamed		6.7	10.4	15.8	1137	1778	2696	32.0	50.1	76.0	1332	2083	3159	37.5	58.7	89.0
Boiled	$TEAC_{FRAP}$	0.4	2.1	7.3	75.6	362	1242	1.3	6.0	20.7	88.6	424	1456	1.5	7.1	24.2
Breaded		1.7	2.8	3.9	290	483	674	4.8	8.0	11.2	340	566	790	5.7	9.4	13.1
Fried		1.5	3.2	7.3	250	551	1238	4.2	9.2	20.6	293	646	1450	4.9	10.7	24.1
Grilled		1.4	2.7	5.8	241	458	982	4.0	7.6	16.3	282	537	1150	4.7	8.9	19.1
Raw		1.5	2.6	4.9	254	451	842	4.2	7.5	14.0	298	529	986	4.9	8.8	16.4
Roasted		1.4	2.2	4.3	236	380	742	3.9	6.3	12.3	276	445	869	4.6	7.4	14.5
Steamed		1.3	2.2	4.6	228	383	783	3.8	6.4	13.0	267	449	917	4.4	7.5	15.2
N	$TEAC_{ABTS}$	5.7	11.1	26.0	970	1898	4430	27.3	53.5	125	1137	2224	5191	32.0	62.7	146
R		5.2	9.3	13.6	892	1581	2323	25.1	44.6	65.5	1045	1853	2722	29.4	52.2	76.7
WD		3.1	10.8	20.5	525	1835	3491	14.8	51.7	98.4	616	2150	4090	17.3	60.6	115
N	$TEAC_{FRAP}$	0.4	2.3	7.3	75.6	392	1238	1.3	6.5	20.6	88.6	459	1450	1.5	7.6	24.1
R		1.5	2.6	4.9	254	451	842	4.2	7.5	14.0	298	529	986	4.9	8.8	16.4
WD		1.3	2.6	7.3	228	439	1242	3.8	7.3	20.7	267	514	1456	4.4	8.5	24.2

N: Normal cooking time; R: Raw (not cooked); WD: Well done.
[a] Considering consumption for a whole year.
[b] Consideing the complete serving ingested a particular day.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2018.12.007.

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